Evaluation of Plant Resistance in Field Pea by Host Plant Choice Behaviour of Pea Weevil (*Bruchus pisorum* L.): Implications for Pest Management

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Cover: Left: adult pea weevil (*Bruchus pisorum*) on a pea flower. Right from top to the bottom: field trial site in Liben, Ethiopia and field pea seeds damaged by pea weevil. (Photo: Esayas Mendesil Amosa)

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Abstract

Field pea (*Pisum sativum* L.) is an important grain legume crop due to its nutritional value and role in improving soil fertility in cropping systems. Insect pests are one of the main production constraints for field pea, with pea weevil (PW), (*Bruchus pisorum* L.) being an economically important pest of field pea worldwide. Current PW control practices rely on chemical insecticides, which are unaffordable for most small-scale farmers in developing countries such as Ethiopia, where PW is established in northern and north-western regions, causing severe crop losses. Furthermore, pesticides have adverse effects on human health and the environment. This thesis investigated host plant resistance in field pea and mapped Ethiopian farmers' knowledge and management practices to control PW. The farmers surveyed were aware of PW and able to identify damaged seeds based on common symptoms, but most considered PW a storage pest. To resolve this knowledge gap, it is important to provide training for these farmers. In addition, development of integrated pest management strategies for PW is vital for sustainable production of field pea. Most Ethiopian field pea accessions that were screened for resistance to PW were found to lack resistance and only a few accessions showed moderate levels of resistance based on percentage seed damage (PSD). Gene bank accessions and newly collected populations performed better than released varieties. Some of the accessions formed neoplasm in the greenhouse due to neoplastic gene (*Np*) and these genotypes had less PSD than non-*Np* genotypes. Ultraviolet light suppressed neoplasm formation in *Np* genotypes, while intercropping of *Np* genotypes with sorghum enhanced neoplasm formation. Female PW use flower volatiles to locate host plants, but no discrimination between genotypes based on flower volatiles was found. However, oviposition patterns reflected the resistance pattern found in field screening. *Adet* was an attractive genotype for oviposition, while non-host plants (*Pisum fulvum* Sibth. et Sm. and *Lathyrus sativus* L.) were less preferred by female PW. Pod morphological traits such as pod wall thickness, trichomes and neoplasm may influence oviposition acceptance by female weevils. These results can be used in developing alternative pest management strategies for PW.

Keywords: Bruchus pisorum, field pea, host selection, IPM, legume, neoplasm, oviposition, pea weevil, plant resistance, trichome

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Dedication

To my family

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Abel Teshome, Esayas Mendesil, Mulatu Geleta, Derege Andargie, Peter Anderson, Birgitta Rämert, Emiru Seyoum, Ylva Hillbur, Kifle Dagne, Tomas Bryngelsson (2015). Screening the primary gene pool of field pea (*Pisum sativum* L. subsp. *Sativum*) in Ethiopia for resistance against pea weevil (*Bruchus pisorum* L.). *Genet Resour Crop Evol* 62, 525-538.

II Esayas Mendesil, Birgitta Rämert, Ylva Hillbur, Peter Anderson (2015). Attraction of pea weevil (*Bruchus pisorum* L.) to field pea flower volatiles. (Manuscript).

III Esayas Mendesil, Birgitta Rämert, Salla Marttila, Ylva Hillbur, Peter Anderson (2015). Oviposition preference of pea weevil (*Bruchus pisorum* L.) among host and non-host plants and its implications for pest management. (Submitted).

IV Abel Teshome, Tomas Bryngelsson, Esayas Mendesil, Salla Marttila, Mulatu Geleta (2015). Enhancing neoplasm expression in field pea (*Pisum sativum* L.) via intercropping and its significance to pea weevil (*Bruchus pisorum* L.) management. (Submitted).

V Esayas Mendesil, Zekarias Shumeta, Peter Anderson, Birgitta Rämert (2015). Smallholder farmers' perspectives on pea weevil and pest management practices in northern and north-western Ethiopia. (Submitted).

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The contribution of Esayas Mendesil to the papers included in this thesis was as follows:

I Designed, conducted and analysed the data and wrote the manuscript together with the co-authors.

II Designed, conducted and analysed the data and wrote the manuscript together with the co-authors.

III Designed, conducted and analysed the data and wrote the manuscript together with the co-authors.

IV Designed and conducted the experiment and contributed to writing the manuscript along with the co-authors.

V Designed and conducted the survey work, analysed the data and wrote the manuscript together with the co-authors.

Abbreviations

FFS	Farmers' field school
HPR	Host plant resistance
HIPV	Herbivore-induced plant volatiles
IPM	Integrated pest management
Np	Neoplastic pod
PW	Pea weevil
PSD	Percentage seed damage
SEM	Scanning electron microscope

1 Introduction

Worldwide crop production is threatened by biotic stresses such as pest insects, plant diseases and weeds, which cause about 40% of crop damage annually (Pimentel, 2009). Insect pests alone are responsible for about 20% of all damage in crop production (Sallam, 1999). As a result of increasing global population growth and corresponding predicted increasing demand for world food grain production, crop losses caused by pest organisms are a threat to food security, particularly in developing countries of the world (Godfray *et al*., 2010; Tilman *et al*., 2011). Reducing damage caused by field and post-harvest pest organisms could contribute strongly to sustainable crop production and food security at large (Nwilene *et al*., 2008; Bruce, 2010). Hence emphasis should be placed not only on increasing crop production, but also on reducing crop losses to pests.

As is commonly the case in developing countries, agriculture is an important economic sector in Ethiopia, accounting for more than 40% of GDP, employing over 85% of the total population and contributing about 90% of export earnings (World Bank, 2012; NBE, 2015). It is predominantly carried out by smallholder farmers. Ethiopia's agro-ecology is highly diversified, making it possible to grow a wide variety of crops such as cereals, legumes, horticultural crops, *etc*. In addition, the country is considered one of the centres of origin of crops such as tef (*Eragrostis tef* (Zucc.) Trotter), niger (*Guizotia abyssinica* (L.f.) Cass.), coffee (*Coffea arabica* L.) and the centre of genetic diversity for *e.g*. field pea (*Pisum sativum* L.), wheat (*Triticum aestivum* L.) and sorghum (*Sorghum bicolor* (L.) Moench) (IBC, 2008).

Field pea is a cool-season legume crop that belongs to the Leguminosae. Although little is known about the exact origin of this crop, it is mainly grown in tropical highlands and in many countries in temperate regions (Cousin, 1997; Messiaen *et al*., 2006). According to a recent FAO report, the major field pea growing country is Canada, followed by the Russian Federation, India,

France and Australia (FAOSTAT, 2012). Total world production of dry peas during 2012 was more than 10 million tonnes, from a total area of almost 7 million hectares (FAOSTAT, 2012). In that year Ethiopia ranked sixth in the world, with total field pea production of more than 300,000 tonnes. Field pea is the second most important grain legume crop in the country, after faba bean (*Vicia faba* L.), and is predominantly grown by small-scale farmers for household consumption and as a source of income.

Field pea is an important crop for both human food and animal feed due to its high nutritional value, as it is rich in protein, carbohydrate and some minerals (Khan & Croser, 2004). Furthermore, it plays an important role in cropping systems by improving of soil fertility due to its symbiotic nitrogen fixing ability (French, 2004; Messiaen *et al*., 2006). As is the case for other grain legumes, insect pests are one of the main production constraints for field pea. In general, over 15 insect species are known to attack field pea, although only a few of these are considered as economically important pests (Clement *et al*., 1994; Sharma *et al*., 2010). In Ethiopia, pea weevil, *Bruchus pisorum* L., pea aphid, *Acyrthosiphon pisum* (Harris), African bollworm, *Helicoverpa armigera* (Hubner) and adzuki bean beetle, *Callosobruchus chinensis* L. are reported to be the major insect pests of field pea (Ali *et al*., 2008).

2 Background

2.1 Pea weevil biology

Pea weevil, *Bruchus pisorum* (Coleoptera: Bruchidae) is a univoltine insect that feeds and reproduces only on *P. sativum*. The adult weevils hibernate in sheltered areas such as grain stores, grain bins, tree bark and debris (Brindley *et al*., 1956). After emerging from hibernation sites, the weevils fly to the pea field guided by the scent of pea flowers (McDonald, 1995). It has been shown that most weevils come from the previous pea crop field and nearby areas (Armstrong & Matthews, 2005).

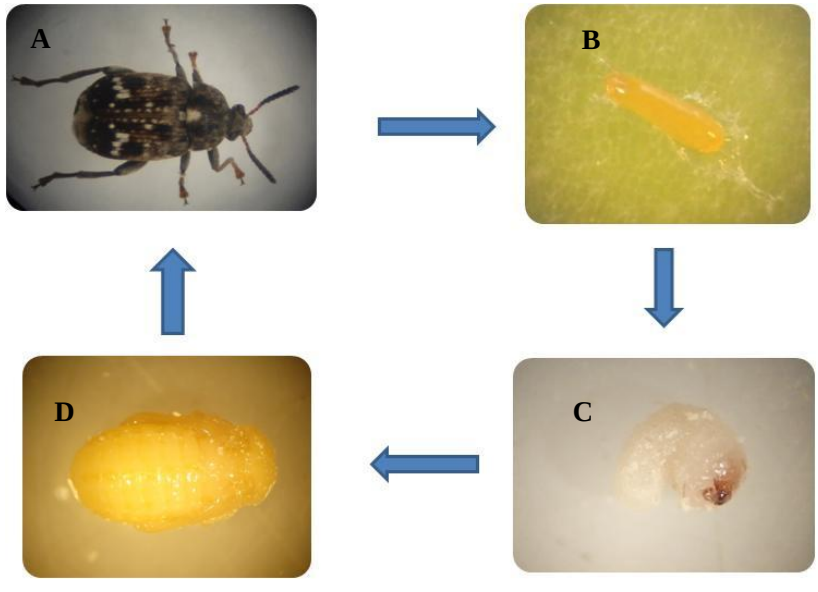

Figure 1. Life cycle of pea weevil. (A) Adult, (B) egg, (C) larva and (D) pupa.

Unlike male weevils, newly emerged females are sexually immature and need to feed on pollen of field pea before commencing oviposition (Pesho & van Houten, 1982). It has been shown that female weevils also feed on pollen of non-host plant species (Pesho & van Houten, 1982; Annis and O'Keeffe, 1984a), suggesting that *P. sativum* is not the only source of pollen for the weevils. After a few days of feeding on pollen, the weevils mate in the pea field. In general, female weevils start oviposition about 2 to 2.5 weeks after their arrival (Baker, 1998). Gravid female weevils lay eggs on living green pods of any size (Brindley *et al*., 1956; McDonald, 1995), but flat and swollen pea pods are the preferred host plant stages (Hardie & Clement, 2001). The incubation period of the eggs ranges from 4 to 14 days, and hatching larvae bore through the pod walls directly to the seed. The larvae develop within the seed, feeding on the seed cotyledon, and complete development either on living plants or after harvest in storage. However, adult weevils never oviposit on dry seeds. The development period of the larvae ranges from 4 to 8 weeks. Pupation takes place inside the seed and lasts for about 12 to 14 days (Brindley and Chamberlin, 1952). Upon completing development, adult weevils emerge from damaged seeds (Figure 1).

2.2 Pest status and control methods for pea weevil

Pea weevil is an economically important pest of *Pisum sativum* that is prevalent in almost all field pea growing countries of the world (Clement *et al*., 2000; Plantwise, 2014) (Figure 2).

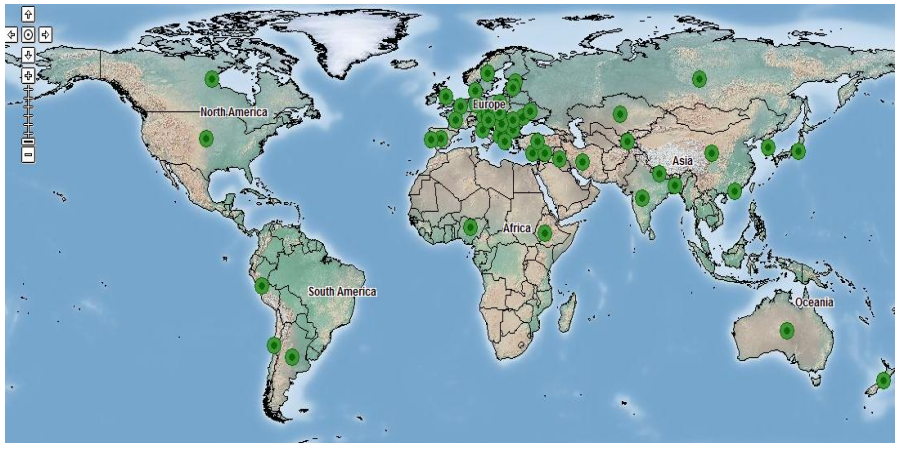

Figure 2. Map showing distribution of pea weevil, *Bruchus pisorum* (Source: Plantwise (2014), with permission from CABI).

In Ethiopia, pea weevil was first reported around the mid-1970s in the north part of the country (Abate, 2006), where it is now well established. The pest has since spread throughout the country, mainly through seed exchange and trading of infested seeds (Ali *et al*., 2008). Damage to the crop is caused by the pea weevil larvae and infested seeds are generally of no value for human food, and animal feed or as seeds for planting (Clement *et al*., 2000; Seyoum *et al*., 2012) (Figure 3). It has been shown that in Ethiopia, pea weevil can inflict up to 85% seed damage and 59% seed weight loss (Teka, 2002; Seyoum *et al*., 2012).

The pea weevil can easily spread from one area to another via infested seeds. At early stages of infestation, attacked seeds can scarcely be detected as there is only a small black dot caused by neonate larval entry on the pea surface. In general, Brindley *et al.* (1956) identified four main sources of pea weevil infestation: (1) Shattered and fallen peas, mainly during harvesting, (2) volunteer peas, which grow from leftover seeds from previous years' harvest, (3) infested seeds in pea hay, and (4) infested pea seeds placed in storage. Knowledge about the sources of infestation is very important when designing pest management strategies.

Figure 3. Field pea seeds damaged by pea weevil.

Current pest management practices for pea weevil worldwide are mainly dependent on the use of chemical insecticide spraying in the field and

fumigation in the store (Horne & Bailey, 1991; Waterford & Winks, 1994; Seidenglanz *et al*., 2011). Several studies screening *P. sativum* accessions for resistance to pea weevil have been reported (*e.g*. Pesho *et al*., 1977; Hardie *et al*., 1995; Clement *et al*., 2002; Ali *et al*., 2008), but the levels of resistance found are generally low (Hardie *et al*., 1995). However, pea weevil resistance has been found in accessions of wild pea (*Pisum fulvum* Sibth. et Sm.) (Hardie *et al*., 1995; Byrne *et al*., 2008; Clement *et al*., 2002; Clement *et al*., 2009) and breeding efforts are underway to transfer this resistance from *P. fulvum* to *P. sativum* (Clement *et al*., 2009). Furthermore, genetically modified field pea that resists pea weevil damage has been developed (Schroeder *et al*., 1995; De Sousa-Majer *et al*., 2007), but concerns have been raised regarding potential health problems associated with these transgenic peas (Prescott *et al*., 2005).

Pea weevils are attacked by several species of natural enemies, such as the hymenopteran egg parasitoids *Uscana senex* Grese (Trichogrammatidae) (Hormazabal & Gerding, 1998) and *U. chiliensis* sp.nov (Pintureau *et al*., 1999), the larval parasitoid *Triaspis thoracicus* (Curtis) (Braconidae) (Brindley *et al*., 1956; Annis & O'Keeffe, 1987), and *Eupteromalus leguminis* Gahan (Pteromalidae) and *Microdontomerus anthonomi* (Crawford) (Torymidae), which are larval/pupal parasitoids (Larson *et al*., 1938). However, biological control strategies of pea weevil have received little attention in the past, indicating a need for thorough studies of these natural enemies to explore their potential as biological control agents for the pea weevil.

2.3 Role of plant chemicals in host location and oviposition behaviour

In nature, insects are exposed to a variety of plant species and selecting the right host plant is a crucial behavioural process that includes location and acceptance of the plant for feeding and site of oviposition, thus involving various sensory systems (Bernays & Chapman, 1994). For location of distant host plants, most herbivorous insects rely on olfactory and visual cues. Based on information obtained from the plant, the insect is either attracted to the plant or repelled (Bernays & Chapman, 1994). Several studies have shown that insect herbivores utilise plant chemical cues such as volatiles released from the plant in order to locate their host plant (*e.g*. Visser, 1986; Bruce *et al*., 2005). Plants normally emit a variety of organic compounds to the environment. An extensive study by Knudsen *et al*. (2006) reported 1719 chemical compounds belonging to seven major compound classes, emitted from 90 plant families.

Upon making contact with the plant, the insect assesses its suitability for feeding and oviposition based on volatiles and contact-chemosensory, tactile

and visual cues (Bernays & Chapman, 1994). Plant morphological traits such as wax layers and trichomes, as well as primary and secondary metabolites, influence the acceptance of the host plant. Consequently, information obtained from the host plant elicits behavioural responses such as feeding and oviposition (Schoonhoven *et al*., 2005). For example, for diamondback moth, *Plutella xylostella* L., which is a pest of Brassicas, isothiocyanates serve as attractants and oviposition stimulants (Furlong *et al*., 2013). The female Mexican bean beetle, *Epilachna varivestis* Mulsant prefers to oviposit on varieties of its host plant, lima bean, *Phaseolus lunatus* L. that have low cyanogenic content compared with varieties with a high content (Ballhorn & Lieberei, 2006). This indicates that plant chemicals influence insects not only to choose the right species of host plant, but also to select a suitable plant from the same species for feeding and oviposition.

2.4 Host plant resistance to insect herbivores

Insect herbivores depend on plants for their nutritional requirement and site of oviposition. Plants, on the other hand, employ morphological traits and chemical compounds against insect herbivory (Kessler & Baldwin, 2002; Smith, 2005). Host plant resistance (HPR) is defined as "the sum of genetically inherited qualities that results in a plant of one cultivar or species being less damaged by a pest arthropod than a susceptible plant lacking these qualities" (Smith & Clement, 2012). Insect-resistant varieties can be used as alternatives to chemical insecticides or in combination with other pest management methods. However, although there has been progress in identifying sources of resistance in grain legumes in the past, little effort has been devoted to insect resistance breeding partly due to the current preference for, and reliance on, chemical insecticides (Sharma & Ortiz, 2002; Sharma *et al*, 2010).

Plant resistance is divided into three categories, namely antibiosis, antixenosis and tolerance. Antibiosis refers to plant resistance due to morphological traits and/or chemical compounds that negatively affect the physiology of an insect (Kogan, 1994; Smith, 2005; Smith & Clement, 2012). The antibiosis resistance of a plant is measured based on parameters such as insect development, reproduction, survival and mortality, and plant damage score (Dent, 2000). In contrast, antixenosis resistance mainly affects the behaviour of the insect, so that a plant exhibiting such resistance is not detected as a suitable host by the insect. Plant morphological traits and/or chemical compounds may be involved in antixenosis resistance (Dent, 2000; Smith, 2005; Smith & Clement, 2012). This type of plant resistance is measured in terms of oviposition rate and number of emigrating insects in a given set of

cultivars (Dent, 2000). Tolerance is the ability of a plant to withstand or recover from insect herbivory without inflicting any adverse effect on the insect (Dent, 2000; Smith & Clement, 2012). The tolerance of a plant to insect infestation can be expressed in terms of yield (Dent, 2000). A review by Smith and Clement (2012) describes plant resistance categories and resistance genes for various crops against different insect pests, indicating progress and increasing interest in development of insect-resistant crops.

Furthermore, plant resistance can be either constitutive or induced after herbivory. Constitutive resistance is the inherent resistance ability of plants that is expressed irrespective of attack by the insect and which negatively affects the insect attacking the plant. Induced resistance is expressed in response to insect herbivory (Schoonhoven *et al*., 2005). Both constitutive and induced resistance can be grouped into direct and indirect types of resistance.

2.4.1 Direct resistance

Plant morphological traits and secondary metabolites play a crucial role in direct resistance to insect damage (Kessler and Baldwin, 2002; Schoonhoven *et al*., 2005). These resistance factors can be developed constitutively without insect attack or expressed after herbivory (Dalin *et al*., 2008). Plant structural defence is defined as "any morphological or anatomical trait that confers a fitness advantage to the plant by directly deterring herbivores from feeding on it" (Hanley *et al*., 2007). Plant morphological traits include surface wax, trichomes and toughness of plant tissues (Bernays & Chapman, 1994). A number of studies have described the role of plant morphological traits in HPR. For example, in field pea, pea weevil and pea leaf weevil, *Sitona lineatus* L. cause more damage to varieties with a reduced wax layer on plants than to normal wax varieties (White & Eigenbrode, 2000; Chang *et al*., 2006). In addition, upon oviposition by female pea weevil, field pea plants with neoplastic gene (*Np*) form 'a postular' outgrowth on the pod surface that reduces larval entry into the pod (Berdnikov *et al*., 1992; Doss *et al*., 2000). Neoplasm also develops when peas with the *Np* gene are grown in the greenhouse, due to the suppression by UV light (Nuttall & Lyall, 1964).

Plants also employ secondary metabolites against insect attack and various studies have demonstrated the importance of secondary metabolites in plant defence (reviewed by Mithöfer & Boland, 2012). These secondary metabolites can be developed constitutively or induced after herbivory (Mithöfer & Boland, 2012). There are overwhelming numbers of secondary metabolites such as terpenoids, alkaloids, phenolics, glucosinolates and cyanogenic glucosides, which play a crucial role in plant resistance to insect attack (Wu & Baldwin, 2010; Mithöfer & Boland, 2012). These chemical compounds act as a

direct defence against insect herbivores in various ways, such as adversely affecting insect growth and reproduction and being toxic and/or repellent to herbivores (Wu & Baldwin, 2010). Trichomes are one of the sites of secretion of secondary metabolites that can be toxic or repellent to insects (Bernays & Chapman, 1994; Dalin *et al*., 2008).

2.4.2 Indirect resistance

Plants attacked by insect herbivores are known to release herbivore-induced plant volatiles (HIPVs) that attract natural enemies (parasitoids and predators). These natural enemies suppress insect herbivores and thereby minimise the damage inflicted on the attacked plants (Kessler & Baldwin, 2002; Schoonhoven *et al*., 2005). Various studies have shown the attraction of parasitoids and predators of insect herbivores caused by emission of HIPVs (reviewed by McCormick *et al*., 2012). In addition, it has been shown that oviposition-induced plant volatiles emitted after egg laying attract egg parasitoids and thereby protect the plant from damage (reviewed by Hilker & Fatouros, 2015). Attacked plants are also known to produce extrafloral nectar that benefits natural enemies and enhances their activities against the pest herbivores (Kessler & Baldwin, 2002).

Although plant morphological traits and chemical compounds play a paramount role in host plant resistance to insect attack, such plant traits are often overlooked during plant breeding programmes that aim to improve a particular trait such as yield. As a result, such important plant traits may be inadvertently lost in the plant breeding process. For example, landrace maize varieties release HIPVs in response to oviposition by stem borer moths, *Chilo partellus* (Swinhoe), but the trait has been lost in commercial maize varieties (Tamiru *et al*., 2011). Furthermore, a recent review by Chen *et al.* (2015) showed the impact of plant domestication on loss of morphological traits and secondary metabolites compared with their wild progenitors. Such findings highlight the importance of paying due consideration to plant traits related to HPR in a breeding programme, as demonstrated by Tamiru *et al.* (2015) in a study on breeding for egg-induced defence traits.

2.5 Integrated pest management

The adverse effects due to the current high dependency on chemical insecticides, such as development of insecticide resistance coupled with increasing public awareness about the effects of pesticides on human health and the environment, have provided the impetus for integrated pest management (IPM) (Kogan, 1998; Dhawan & Peshin, 2009). There are

multiple definitions of IPM; *e.g*. a review by Bajwa & Kogan (2002) documented 67 different definitions. According to Dent (1995), IPM is "a pest management system that in the socioeconomic context of farming systems, the associated environment and the population dynamics of the pest species, utilizes all suitable techniques in as compatible manner as possible and maintains the pest population levels below those causing economic injury." IPM is thus a pest management approach that employs different tactics in a combined approach to keep the pest population below the damaging level. This includes cultural control, biological control, host plant resistance, semiochemicals and chemical control with insecticides. The benefits of IPM are not limited to pest management, but also include the environment and society (Dhawan & Peshin, 2009).

Although insect pests are considered the main biotic production constraint for grain legumes in the tropics (Edwards & Singh, 2006; Sharma *et al*., 2010), the available IPM strategies for grain legume pests are very limited (Clement *et al*., 2000). There have been some efforts to develop IPM strategies for some pests of legume crops (*e.g*. Sharma, 2006). For an IPM programme to be successful, involvement of farmers in the design and implementation of IPM measures is crucial (Shepard *et al*., 2009). This can be achieved through *e.g*. farmers' field schools (FFS). The success of IPM-FFS in Asia in minimising dependency on chemical pesticides and in improving crop productivity has become a model for other countries (Pontius *et al*., 2002).

3 Objectives

The main objectives of the work described in this thesis were to:

1. Screen Ethiopian field pea accessions for resistance to pea weevil (Paper I).
2. Determine the attraction of pea weevil to flower volatiles of field pea genotypes (Paper II).
3. Examine oviposition preferences of pea weevil among host and non-host plants and determine the influence of pod morphological traits on oviposition by the weevil (Paper III).
4. Study the effects of UV light and intercropping on the formation of neoplasm by pods of field pea genotypes (Paper IV).
5. Assess farmers' knowledge and current pest management practices being used to control pea weevil in Ethiopia (Paper V).

4 Experimental approaches

4.1 Field and greenhouse experiments

The screening of Ethiopian field pea germplasm for resistance to pea weevil was conducted under both field and greenhouse conditions. Initially, a total of 602 field pea accessions, including gene bank accessions, released varieties and new collections, were evaluated at three different locations in northern and north-western Ethiopia: Liben (11°50'N, 37°10'E), Ebinat (12°10'N, 38°50'E) and Sekota (13°00'N, 38°50'E) (Figure 4).

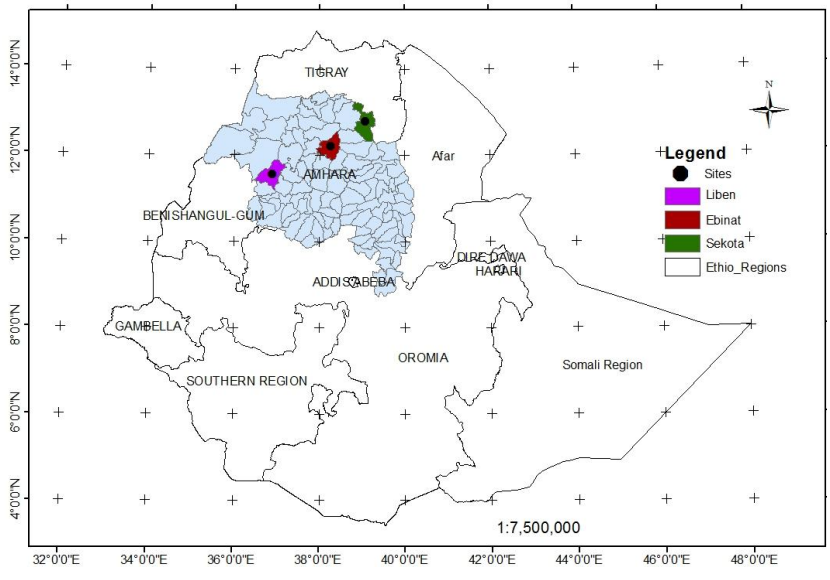

Figure 4. Geographical position of the three experimental sites in Ethiopia used for evaluation of field pea accessions.

These study sites were selected based on previous reports of high and consistent infestation by pea weevil in these areas. Based on results obtained from the first field trial, 100 genotypes were selected for a second round of experiments at two sites in Liben, one of the previous year's trial sites (Figure 4). Harvested seed samples of each accession were evaluated for their resistance to pea weevil based on percentage seed damage (PSD). In addition, seed colour and shape of the accessions were recorded.

Furthermore, three consecutive greenhouse experiments were performed at the Swedish University of Agricultural Sciences in Alnarp to evaluate the level of resistance of genotypes selected from nine accessions selected from field experiments. These test plants were grown in a greenhouse chamber (22 ^{o}C and minimum of 12 h light) and artificially infested by newly emerged adult weevils in insect cages (60 cm x 60 cm x 120 cm, MegaView Science Co Ltd, Taiwan). The level of resistance of test genotypes was determined based on PSD.

Screening of neoplasm-producing genotypes (*Np*) was conducted in the greenhouse following the above procedures. In addition, the effect of UV light on the neoplasm formation was studied in five chambers in the greenhouse. One of the chambers was a control (without UV light). Of the remaining four chambers, two had one UV lamp (3U 15W UV light bulb) and the other two had two UV lamps (2 x 3U 15W UV light bulbs). Test plants were exposed to UV light for 12 hours. After harvesting of peas, the level of neoplasm formation was recorded. In addition, field experiments were carried out in Alnarp to study the formation of neoplasm in *Np* genotypes intercropped with, and thus shadowed by, sorghum.

4.2 Attraction and oviposition bioassay

The attraction of adult pea weevils to flower volatiles of field pea genotypes was tested using a two-choice olfactometer in a climate chamber (24 ^{o}C, 60% RH and 12:12 light/dark cycle). Three field pea genotypes with different levels of pea weevil resistance were used in this study: *Adet* (susceptible) and *235899-1* and *32410-1* (both moderately resistant). All plants were grown in a biotron chamber (22 ^{o}C, 75% RH and 12:12 h light/dark cycle). Newly emerged weevils from infested pea seeds were used for this experiment. Attraction of male and female weevils to flower volatiles of the three field pea genotypes was compared with their attractions to clean air. In addition, attraction of male and female weevils to volatiles of susceptible and moderately resistant genotypes was compared. Finally, the influence of feeding

status on the attraction of female weevils to the three pea genotypes was studied.

Oviposition acceptance by female pea weevils was studied both in no-choice and dual-choice bioassays. The experiments were performed in a climate chamber at 24 ^{o}C, 60% RH and a 12:12 h light/dark cycle. A pair of newly emerged male and female weevils was introduced into an insect rearing cage (31 cm x 22.5 cm x 12 cm). Prior to the experiments, female weevils were fed with field pea pollen for 10 days. The oviposition bioassay was conducted according to the methods of Hardie and Clement (2001) and Clement *et al*. (2002) with some modifications. In the no-choice test, two flat pods of one genotype or species (either *Adet*, *2358991-1*, *32410-1*, *P. fulvum* or *Lathyrus sativus* L.) were provided for oviposition to each weevil in a cage. In the dual-choice test, one pod of *Adet* (control) and one pod from one of the other four genotypes/species were provided to the weevil in a cage. Oviposition bioassays were conducted over 10 days, with each experimental setup replicated 10 times.

4.3 Morphological characteristics

The pod wall thickness of host and non-host test genotypes was measured using an Absolute Digimatic Caliper (500-182-30, Mitutoyo, Japan). For these measurements, a total of 40 green pods were sampled from 10 plants of each genotype. In order to examine whether there were any differences in the pod anatomy of host and non-host plants, green pod samples were examined using a scanning electron microscope (435VP, LEO Electron Microscopy Ltd., Cambridge, UK) with 10 kV.

4.4 Survey to determine farmers' knowledge and management practices

A survey of 400 field pea growers was conducted in four main field pea growing districts (Semen Achefer, Ylmana Densa, Ebinat and Farta) in northern and north-western Ethiopia. The survey comprised interviews with a structured questionnaire and focus group discussions with selected farmers. The data collected included household demographic characteristics, farm characteristics, pea weevil problems, farmers' knowledge about pea weevil and current pest management practices. Both descriptive statistics and econometric tools, such as a binary logit model, were used to analyse the data.

5 Summary of results

5.1 Plant resistance in field pea genotypes (Papers I and IV)

In the screening of field pea accessions for resistance to pea weevil, the mean PSD ranged from 12% to 98% (Figure 5). Ebinat had the highest PSD (52.2%), followed by Liben (46.5%) and Sekota (29.7). Most of the accessions had high PSD and only nine of the accessions showed moderate level of resistance to pea weevil (PSD values <20%). In general, the gene bank accessions and newly collected populations were less damaged than the released varieties, suggesting that the latter varieties are more susceptible to the weevil. It has been shown in other crops that the susceptibility of improved varieties is associated with the loss of resistance traits during breeding for specific traits such as high yield (Keneni *et al*., 2011; Tamiru *et al*., 2011).

During the second year, 100 accessions were screened at two sites in Liben. Only three of these accessions showed low PSD (<30%), while the remaining accessions were highly attacked by the weevil. In general, most of the accessions during the second year of screening had higher damage than was recorded in the first year of screening. The weevils from shattered and volunteer peas might have contributed to increased infestation by the weevil (Brindley *et al*., 1956) in the second year of screening. It was also found that some genotypes selected from accessions *226037*, *236413* and *32410* showed lower PSD compared with the susceptible genotypes used in the greenhouse experiment. Previous screenings of field pea accessions for resistance to pea weevil (*e.g*. Pesho *et al*., 1977; Hardie *et al*., 1995; Clement *et al*., 2002; Gantner *et al*., 2008; Ali *et al*., 2008) have demonstrated an absence of resistance in the primary gene pool of field pea (Hardie *et al*., 1995; Clement *et al*., 2002). It is possible that field pea accessions/genotypes which showed a moderate level of resistance to the weevil can be used to control pea weevil

under small-scale farming conditions, in combination with other pest control methods in IPM strategies (*e.g*. intercropping).

Figure 5. Field trial site in Liben, Ethiopia.

Among the field pea genotypes tested in experiments, four (*32433*, *235899*, *226037* and *237065*) consistently displayed the formation of pod neoplasm in the greenhouse. These *Np* genotypes also had lower average PSD, ranging from 12% to 18%. It has been shown previously that neoplasm is formed on field pea plants with the *Np* gene due to either abiotic factors, *i.e*. under suppressed UV light (Nuttall & Lyall, 1964; Snoad & Matthews, 1969), or oviposition by female pea weevil as an induced defence response (Berdnikov *et al*., 1992; Doss *et al*., 1995; Doss *et al*., 2000).

The F_1 hybrids (crosses between non-neoplastic pollen recipients and neoplastic pollen donors) expressed neoplasm formation in the greenhouse, indicating heritability of this trait. This corroborates earlier findings by Nuttall and Lyall (1964). The UV light experiments revealed a low percentage of neoplastic pods in UV chambers compared with non-UV chambers (control), confirming the suppression of neoplasm expression under UV light shown in earlier studies (Nuttall & Lyall, 1964; Snoad & Matthews, 1969). In addition,

intercropping of *Np* genotypes with sorghum shading the pea plants, and thus reducing UV light, resulted in about 30% neoplastic pods. This raises the prospect of intercropping to enhance neoplasm expression under field conditions and thereby the possibility to minimise damage caused by the weevils using this morphological trait.

5.2 Attraction and oviposition behaviour of pea weevil (Papers II and III)

In a two-choice olfactometer bioassay, adult pea weevils were attracted to field pea volatiles, demonstrating the role of flower volatiles in long-range attraction of the weevil to field pea plants. The need for newly emerged female weevils to feed on pollen before commencing oviposition (Pesho & van Houten, 1982) also underlines the importance of pea flowers for female weevils. The weevils were attracted to volatiles of both susceptible (*Adet*) and moderately resistant genotypes (*235899-1* and *32410-1*) compared with clean air. This indicates that flower volatiles may not be important for discrimination between genotypes and thus other plant cues might affect the females' choice. Regardless of their feeding status, female weevils were attracted to both susceptible and moderately resistant genotype.

Figure 6. Plants used in oviposition experiments. (A) *Pisum sativum*, (B) *Pisum fulvum* and (C) *Lathyrus sativus*.

In the oviposition bioassays, three field pea genotypes and two non-host leguminous plants (*Pisum fulvum* and *Lathyrus sativus*) (Figure 6) were included to determine oviposition acceptance by the pea weevil. Female weevils were able to discriminate between host and non-host genotypes. Both in no-choice and dual-choice bioassays, female weevils consistently laid more eggs on *Adet* than on the other host genotypes and non-host plants. This is in

agreement with results of field experiments where *Adet* showed the highest PSD (Teshome *et al*., 2015). It indicates that oviposition acceptance of the weevil is consistent with the performance of field pea genotypes to pea weevil damage under field conditions. The high attraction of the weevils to *Adet* demonstrated the potential of this variety as a candidate trap crop. However, since *Adet* considerably increases pea weevil population in the field, it could be grown and then treated with insecticides to make it a 'dead-end trap crop', as suggested by Shelton and Nault (2004) and Shelton and Badenes-Perez (2006).

The females laid very few eggs on the two non-host plants. Earlier studies showed that reduced oviposition on *L. sativus* might be attributable to deterrent compounds on the pods (Jermy & Szentesi, 1978; Annis & O'Keeffe, 1984b). Paper III showed that the formation of neoplasm on pods of *235899-1* (*Np*; Figure 7A), significantly reduced the number of eggs laid on this genotype. The degree of neoplasm formation was negatively correlated with the number of eggs laid per pod, which further confirms the influence of the neoplastic trait on oviposition behaviour of female pea weevil. Earlier studies also demonstrated the importance of this trait in reducing pea weevil damage (Berdnikov *et al*., 1992; Doss *et al*., 1995; Doss *et al*., 2000).

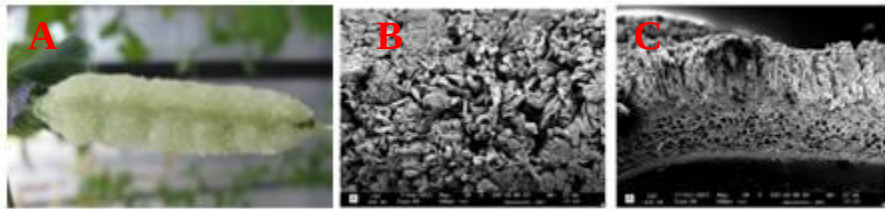

Figure 7. Neoplasm on pod of *Np* genotype of field pea plant grown in the greenhouse. (A) Photo of neoplasm formation on external pod surface, (B) scanning electron micrograph of external pod surface and (C) scanning electron micrographs of cross-section of pod wall.

In an attempt to determine the role of pod morphological traits on oviposition behaviour of the weevil, the pod morphology of the genotypes used was examined in the oviposition experiments. Measurement of pod wall thickness showed that *Np* genotype and *P. fulvum* had thicker pod wall than the other genotypes tested. The thicker pod wall might have partly contributed to the reduced rate of oviposition on these genotypes. A SEM study showed detailed pod anatomical features of the test genotypes. There were occasional hairy trichomes on the pod surface of *32410-1*. In addition, the pod surface of *P. fulvum* and *L. sativus* had glandular trichomes. The *Np* genotype also

showed neoplastic outgrowths that looked like trichome filaments (Figure 7B, C). Such plant morphological traits observed on host and non-host genotypes might have contributed to the reduced oviposition rate observed on these genotypes.

5.3 Ethiopian farmers' perspectives on pea weevil and pest management practices (Paper V)

A survey of 400 field pea growers in four districts of northern and north-western Ethiopia revealed that pea weevil was the main production constraints for field pea. Most of the farmers had knowledge about pea weevil. Analysis by the logit model showed that farmers' knowledge of pea weevil was influenced by the gender of the farmer, farming experience and membership of a cooperative. Farmers reported that they identify pea weevil-damaged seeds based on common symptoms observed on attacked seeds, namely 'sting', 'window' and 'weevil exit hole'. However, the majority of the farmers identify damaged seeds by the 'weevil exit hole', which is clearly noticeable in the store. This might be the reason why most of the farmers surveyed considered pea weevil to be a storage pest. Moreover, while most of the farmers were aware of pea weevil and the damage it causes, the majority of them did not have knowledge about the source and means of spread of the weevil in their farm and village. As a result, most of them did not apply measures to prevent spread and carryover of the weevil from one cropping season to another.

Farmers in the survey districts reported that they used different cultural practices and chemical insecticides for the control of pea weevil. However Paper V also showed that cultural practices such as intercropping and crop rotation did not help to minimise the damage caused by the weevils, mainly due to lack of coordination among farmers in implementing these cultural practices. Due to the flight of the weevils, coordination among farmers in the same area is essential for such cultural practices to be effective (Bajwa & Kogan, 2004). A majority of the farmers reported using insecticides, namely Phostoxin (fumigant) and Actellic powder, in their crop store. However, Paper V showed that there were knowledge gaps concerning application of chemical insecticides, such as the use of non-recommended, expired and adulterated insecticides and poor grain storage system. As a result, farmers often complained about low efficacy of the insecticides they had applied to control pea weevil. Various studies have shown the problems associated with pesticide use in developing countries, such as exposure to pesticide risks and development of insecticide resistance (Kamanula *et al*., 2011; Pretty and

Bharucha, 2015; Khan *et al*., 2015), which underlines the need for training farmers in application of pesticides.

Some of the farmers' cultivation practices, such as sowing pea weevil infested seeds, late harvest and poor storage practices enhanced infestation and carryover of the weevil to the next cropping season. However, if cultivation practices are implemented properly (*e.g*. sowing pea weevil-free seeds), it is possible that they can help to minimise the damage caused by the weevil. In general, lack of adequate knowledge about the pea weevil is an impediment to controlling this pest effectively. Therefore, in order to bridge the knowledge gap identified in this study it is crucial to provide training for farmers, *e.g*. through farmers' field schools.

6 Conclusions and future prospects

Most of the field pea accessions evaluated for resistance to pea weevil in this thesis were found to be susceptible to the weevil, as reflected by their high PSD value. However, a few accessions demonstrated moderate levels of resistance to pea weevil attack. In general, gene bank accessions and newly collected populations performed better than released varieties in terms of pea weevil resistance. The high PSD value of improved varieties could be attributable to loss of resistance ability of these varieties during breeding for other traits, *e.g*. high yield. The moderate levels of resistance found in this thesis might be valuable for smallholder farmers, by minimising damage caused by pea weevil when used *e.g*. in cultivar/genotypic mixtures and in combination with other pest management methods.

It was found that some genotypes possessed the *Np* gene, which is responsible for formation of neoplasm on the pod surface of peas grown under greenhouse conditions. It was demonstrated that this trait is heritable. Furthermore, *Np* genotypes were less attacked by weevils than non-*Np* genotypes (susceptible checks). Similarly, in oviposition bioassays the weevils laid fewer eggs on *Np* genotypes and the number of eggs laid was negatively correlated with the degree of neoplasm formation on pea pods, suggesting the potential of this trait in reducing pea weevil damage. The results presented here and in previous studies indicate that neoplasm formation is inhibited by UV light, which makes it difficult to use genotypes with this trait under field conditions. However, this thesis also demonstrated that intercropping of *Np* genotypes with sorghum plants, providing shade, enhanced the formation of neoplasm under field conditions. Intercropping could thus be a way of exploiting the *Np* trait in the control of pea weevil.

This thesis showed that field pea flower volatiles are important in the host location behaviour of pea weevil. Although females did not discriminate between different genotypes based on floral volatiles, they were able to

discriminate between host and non-host genotypes during oviposition. *Adet* was a highly attractive host genotypes for egg laying, while the other two host genotypes tested had intermediate numbers of eggs, confirming the level of resistance found under field studies. Non-host leguminous plants (*P. fulvum* and *L. sativus*) were the least preferred for oviposition by the weevils. Combining *Adet* either with non-host plants or with *Np* genotypes reduced the total number of eggs laid by the female weevils. This reduced rate of oviposition on non-host plants could be partly attributable to pod morphological traits, such as pod wall thickness and trichomes, that might have an influence on oviposition acceptance by the weevils.

The survey results confirmed that pea weevil is established and threatening field pea production in major field pea growing areas in northern and north-western Ethiopia. Most of the farmers surveyed were aware of pea weevil and able to identify damaged seeds based on common symptoms. However, the majority of the farmers were only able to identify damaged seeds by the 'weevil exit hole', which is more visible in stored peas. Most of the farmers therefore considered pea weevil to be a storage pest, which may hamper efforts to control this pest in the field. This knowledge gap about the weevil should be addressed through providing training for the farmers.

Most of the farmers practiced crop rotation and intercropping for different purposes, for example intercropping of field pea with cereals for soil improvement. This thesis showed that these practices did not contribute to reduce pea weevil damage, partly due to lack of coordination among farmers in the same area. The results also revealed that some of the cultural practices currently used, such as sowing weevil infested seeds, late harvesting and poor storage conditions, enhance the spread and carryover of the weevils to the next cropping season. This highlights the need to train farmers on cultural methods of pest control so as to reduce the damage inflicted by the weevils. Most of the farmers surveyed used chemical insecticides in the store to control weevils, but the majority complained about low efficacy of the pesticides. It is possible that improper application methods and use of non-recommended, expired or adulterated pesticides may contribute to this unsatisfactory control of pea weevil. It should be noted that improper use of pesticides exposes the farmers to pesticide risks. Such problems further underscore the need to train farmers not only on cultural practices, but also on proper use of pesticides. Regulation of pesticides by regional or federal authorities also appears necessary.

Future research on pea weevil control should focus on habitat management strategies, such as intercropping of field pea with non-host plants, *e.g. Lathyrus sativus* (grass pea). It is also important to evaluate other non-host plants that might have the potential to reduce infestation by the weevils. Due to

accessions/genotypes differences in susceptibility to pea weevil damage, it might also be worthwhile studying cultivar/genotypic mixtures of peas as a possible component of pea weevil management strategies. Furthermore, trap cropping, mainly using highly attractive genotypes such as *Adet*, should be tested. Since *Adet* considerably increase pea weevil population in the field study, it would have to be treated with insecticides to make it a dead-end-trap-crop. Development of push-pull strategies is another potential area which should be considered for future research.

Little is known about the role of semiochemicals for pea weevil control and further studies are needed. Semiochemicals, *i.e*. kairomones, sex pheromones and blends thereof (*e.g*. Bruce *et al*., 2011), could be used in monitoring and mass trapping of pea weevil. The potential of natural enemies for control of pea weevil should also be explored further. Another area that needs attention is post harvest: Improved storage systems, *e.g*. metal silos and hermetic plastic bags, have been proven to reduce damage by storage pests without applying pesticides. Given that about 70% of pea losses occur after harvest, improved storage methods are important to control losses. Offering training for smallholder farmers in hotspot areas for pea weevils is essential in order to bridge the knowledge gap identified during the survey. A participatory approach involving farmers in the development of future IPM strategies for control of pea weevil is also strongly recommended.

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